SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

AD-A206 732

ELECTE
MAR 13 1989
D

1b RESTRICTIVE MARKINGS	
3 DISTRIBUTION / AVAILABILITY OF REPORT	Approved for public release; distribution unlimited.
4 PERFORMING ORGANIZATION REPORT NUMBER(S)	5 MONITORING ORGANIZATION REPORT NUMBER(S) AFOSR-TR. 89-0404
6a NAME OF PERFORMING ORGANIZATION: Kent State University	6b OFFICE SYMBOL (If applicable):
7a NAME OF MONITORING ORGANIZATION: Air Force Office of Scientific Research/NL	
6c ADDRESS (City, State, and ZIP Code): Kent, OH 44242	7b ADDRESS (City, State, and ZIP Code): Building 410, Bolling AFB, DC 20332-6448
8a NAME OF FUNDING / SPONSORING ORGANIZATION: AFOSR	8b OFFICE SYMBOL (If applicable): NL
9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: AFOSR-86-0045	
8c ADDRESS (City, State, and ZIP Code): Building 410, Bolling AFB, DC 20332-6448	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 61102F; PROJECT NO. 2312; TASK NO. A2; WORK UNIT ACCESSION NO.

11 TITLE (Include Security Classification)
Description and Manipulation of Membrane Lipid Alterations Associated with Synaptic Functions in Isolated Cerebellar Glomeruli

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13a TYPE OF REPORT	13b TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15 PAGE COUNT
Final	FROM 01 Jan 86 TO 31 Dec 88	Feb 17, 1989	7

16 SUPPLEMENTARY NOTATION

17 COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	

19 ABSTRACT (Continue on reverse if necessary and identify by block number)

Isolated cerebellar and hippocampal mossy fiber synaptosomes were used to assess the relationships between membrane lipid metabolism and the evoked release of excitatory amino acid neurotransmitters. Mossy fiber terminals were radio-labeled with arachidonic acid and the effects of membrane depolarization and calcium influx on the labeling of the component lipid pools were determined. It was observed that depolarization and Ca^{2+} influx stimulated the accumulation of unesterified arachidonate. This effect was correlated with increased production of prostaglandins, and lipoxygenase inhibitors. The depolarization and arachidonate-induced transmitter release was mimicked by the addition of PGE$_{2\alpha}$ and blocked by the cyclooxygenase inhibitor ibuprofen. The correlation of arachidonic acid metabolism with prostaglandin systhesis, ATPase inhibition and Ca^{2+} mobilization, suggests they may all play a role in the mechanisms of neurotransmitter release.

20 DISTRIBUTION / AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION	
☒ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	UNCLASSIFIED	
22a NAME OF RESPONSIBLE INDIVIDUAL: Dr. William O. Berry	22b TELEPHONE (Include Area Code): (202) 767-5021	22c OFFICE SYMBOL: NL

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

89 4 12 044

FINAL TECHNICAL REPORT

TITLE: DESCRIPTION AND MANIPULATION OF MEMBRANE LIPID ALTERATIONS ASSOCIATED WITH SYNAPTIC FUNCTIONS IN ISOLATED CEREBELLAR GLOMERULI

GRANT #: AFOSR-86-0045

PRINCIPAL INVESTIGATOR: Robert V. Dorman

DATE: FEBRUARY 17, 1989

SUMMARY

Isolated cerebellar and hippocampal mossy fiber synaptosomes were used to assess the relationships between membrane lipid metabolism and the evoked release of excitatory amino acid neurotransmitters. A variety of metabolic parameters were investigated, in order to develop a comprehensive model for the mechanisms of transmitter release. Mossy fiber terminals were radiolabeled with [^{3}H]arachidonic acid and the effects of membrane depolarization and calcium influx on the labeling of the component lipid pools were determined. It was observed that depolarization and Ca^{2+} influx stimulated the accumulation of unesterified arachidonate. This effect was correlated with increased production of prostaglandins, in particular PGF_{2a} and PGE_2 and could be blocked with lipase inhibitors, Ca^{2+} channel blockers and chelators. Prostaglandin production was also blocked by cyclooxygenase and lipoxygenase inhibitors. It was determined that the sources of unesterified arachidonate included the choline, ethanolamine and inositol glycerophospholipids and that arachidonate accumulation was related to the evoked release of preloaded D-[^{3}H]aspartate from cerebellar mossy fiber terminals. The depolarization- and arachidonate-induced transmitter release was mimicked by the addition of PGF_{2a} and blocked by the cyclooxygenase inhibitor ibuprofen. We have also observed that exogenous arachidonate stimulates the release of endogenous glutamate from mossy fiber terminals. This effect may be related to the arachidonate-dependent inhibition of Na^+-K^+-ATPase and mobilization of intraterminal free Ca^{2+}. Thus, we have been able to correlate arachidonic acid metabolism with prostaglandin synthesis, ATPase inhibition and Ca^{2+} mobilization, which may all play a role in the mechanisms of neurotransmitter release.

RESEARCH OBJECTIVES

The general objective of this research was to examine the role that membrane lipids play in the mechanisms related to neurotransmitter release from a central synapse. Mossy fiber synaptosomes were isolated from cerebellar cortex, because they are involved in motor control. Similar terminals were also prepared from the hippocampus, because they play a role in learning and memory. In order to investigate the relationships between lipid metabolism and synaptic functions, some specific objectives were outlined.

Specific Objectives

1. Use [^{3}H]arachidonic acid to radiolabel nerve terminal lipids.

2. Determine the effects of membrane depolarization on arachidonate metabolism.

3. Assess the importance of external calcium on resting and depolarization-induced changes in arachidonate metabolism.

4. Investigate the possible mechanisms of arachidonate accumulation in response to depolarizing conditions.

5. Measure prostaglandin accumulation in response to membrane depolarization: assess effects of calcium antagonists and metabolic inhibitors.

6. Correlate the observed alterations in arachidonate and prostaglandin metabolism with the actual release of neurotransmitters. (collaboration with Dr. David M. Terrian, USAF/SAM)

7. Investigate the mechanisms of arachidonate-induced neurotransmitter release: determine its effects on Ca^{2+} mobilization and Na^+-K^+-ATPase activities.

STATUS OF THE RESEARCH

The experimental work carried-out in the past three years was designed to fit within the specific objectives listed above. A summary of the research follows.

1. Radiolabeling of glomerular lipids with [^{3}H]arachidonate:

Attempts were made to isotopically label cerebellar mossy fiber terminals in vivo. We found that arachidonate was incorporated into complex lipids and the uptake was linear with time, up to two hours. However, we also found that the degree of incorporation was low and we would need excessive amounts of isotope in order to obtain enough labeling for the planned experiments. Since then, we have employed an in vitro method for isotope incorporation. We found that the presence of 2-4 uCi of [^{3}H]arachidonate for 15 min provided for optimal labeling of the nerve terminal membranes. This method was then used for the studies of arachidonate metabolism.

2. Effects of membrane depolarization on arachidonate metabolism in the isolated cerebellar mossy fiber synaptosomes:

Nerve terminals lipids were radiolabeled in vitro as described above. The labeled membranes were then used to assess the effects of membrane depolarization on the metabolic flux of arachidonate within the various lipid pools. We found that 45 and 90 mM KCl stimulated a release of arachidonate from the phospholipids and triglycerides. Most arachidonate was released from the choline and inositol glycerophospholipids. Veratradine and veratrine, which induce the influx of Na^+, also stimulated arachidonate accumulation, but to a lesser extent. We suggest that the accumulation of unesterified arachidonate is an important response of nerve terminal membranes to depolarization and that such an effect is related to stimulus-secretion coupling.

3. Role of calcium in depolarization-induced arachidonate accumulation:

It is accepted dogma that the release of neurotransmitters requires the presence of external calcium. Therefore, we investigated the involvement of calcium in the evoked release of arachidonate. We found substantial inhibition of arachidonate accumulation in depolarized nerve terminals when Ca^{2+} was omitted from the incubation medium. We also found that verapamil, a Ca^{2+} channel blocker, inhibited the evoked release of arachidonate. The importance of external calcium for arachidonate accumulation was substantiated when the calcium ionophore A23187 was used to mimic the depolarization-induced effects on arachidonate metabolism when only the ionophore and external calcium were present. We observed no effect of A23187 alone. We interpret these results to show that the depolarization-induced accumulation of arachidonate is a calcium-dependent process.

4. Mechanisms of arachidonate accumulation:

We attempted to determine the biochemical pathways involved in the stimulated accumulation of arachidonate. There is some controversy, especially related to platelet aggregation and secretion processes, about the

exact mechanisms used to produce unesterified arachidonate. Therefore, we exposed depolarized nerve terminals to dibucaine and mepacrine, since these two compounds have been reported to be specific inhibitors of phospholipase A_2. Although both compounds inhibited arachidonate accumulation, neither acted as a specific A_2 inhibitor. So, we will have to wait until truly specific inhibitors are developed and made available before we can accurately assess the biochemical pathways involved in the evoked release of arachidonate.

5. Prostaglandin production by cerebellar mossy fiber synaptosomes:

It has been suggested by ourselves and others that prostaglandins play some role in the evoked release of neurotransmitters from nerve terminals. We (Dr. Terrian et al.) have shown that arachidonate and prostaglandins do stimulate the release of preloaded D-[^{3}H]aspartate. We had previously found that there is an accumulation of arachidonate in stimulated terminals and it was also necessary to determine if the same synaptosomes can convert the arachidonate to prostaglandins. We developed radioimmunoassay procedures for measuring prostaglandin levels. Our results were quite positive, since we found that during a ten min incubation, the presence of 45 mM K^+ stimulated prostaglandin production by 20% when compared to unstimulated controls.

6. Correlations between observed alterations in lipid metabolism and the evoked release of neurotransmitters:

The unique and important attribute of this project is based on the collaborative efforts with Dr. David M. Terrian. We are provided with an opportunity to integrate information derived from two distinct, yet related, programs in order to obtain a more accurate assessment of the mechanisms of neurotransmitter release. We are able to correlate the observed changes in membrane metabolism with the ultimate effect, the actual evoked release of neurotransmitters. This has provided us with the ability to make rapid advances in our understanding of release mechanisms. A summary of some of the correlative studies follow.

First, we know that membrane depolarization does evoke the release of neurotransmitters and the accumulation of unesterified arachidonate and both of these events depend on the presence of external calcium. Second, we found that the presence of exogenous arachidonate causes a dose-dependent release of neurotransmitter and this process is calcium-independent. The fact that the arachidonate-stimulated neurotransmitter release is calcium-independent has led us to suggest that the calcium requirement for transmitter release may be related to the accumulation of arachidonate. Third, we found that inhibition of the conversion of free arachidonate to prostaglandins with ibuprofen also inhibited the evoked release of D-[^{3}H]aspartate. Fourth, we observed that exogenous prostaglandin F_{2alpha} also stimulated a dose-dependent release of transmitter and we now know that these nerve terminals do make this PGF_{2alpha} and its production is stimulated by membrane depolarization.

7. Arachidonic acid and the evoked release of endogenous glutamate.

The effects of membrane depolarization and exogenous arachidonate on the release of L-glutamate from cerebellar and hippocampal mossy fiber terminals were investigated in collaboration with Dr. Terrian. We observed that the terminals release glutamate in response to membrane depolarization with K^+. This effect was dose-dependent. A similar dose-dependent effect was observed

in the presence of added arachidonate. Therefore, depolarization and arachidonate stimulate neurotransmitter release in a fashion similar to that observed for the release of preloaded D-[^{3}H]aspartate. However, some differences were noted. In particular, the presence of cyclooxygenase inhibitors blocks the release of the radiolabeled aspartate, but actually potentiates the release of endogenous glutamate. This discrepency may be related to the nonphysiological distribution of the D-[^{3}H]aspartate and further points to the importance of measuring the secretion of endogenous neurotransmitters. Regardless, the arachidonate-induced release of glutamate from both cerebellar and hippocampal mossy fiber terminals suggests a key role for this fatty acid in the evoked release of neurotransmitters.

8. Mechanisms of arachidonate-evoked neurotransmitter release:

The release of transmitters depends on rapid and reversible movements of ions across the nerve terminal membrane. We have examined the effects of arachidonate on Ca^{2+} mobilization and Na^+-K^+-ATPase, in order to establish a relationship between this fatty acid and ionic currents within the synaptosomes. We observed that both depolarization with K^+ and added arachidonate stimulate the dose-dependent accumulation of intraterminal Ca^{2+}. Exogenous arachidonate also causes a marked inhibition of Na^+-K^+-ATPase. It appears, therefore, that the depolarization-induced accumulation of unesterified arachidonate can cause changes in ion movements similar to those required for neurotransmitter release.

9. The working hypothesis:

The above information has been used to develop a working hypothesis related to lipid metabolism and the secretion of endogenous neurotransmitters. We suggest that membrane depolarization stimulates the Ca^{2+}-dependent accumulation of unesterified arachidonic acid and this fatty acid induces the membrane-dependent events (eg. ion movements, protein phosphorylation, cytoskeletal organization) required for transmitter release.

PUBLICATIONS SUPPORTED BY AFOSR 86-0045:

Manuscripts:

1. Dorman, R.V., Schwartz, M.A. and Terrian, D.M. (1986) Prostaglandin involvement in the evoked release of D-aspartate from cerebellar mossy fiber terminals. Brain Res. Bull. 17:243-248.

2. Terrian, D.M., Bischoff, S.B., Schwartz, M.A. and Dorman, R.V. (1987) Molecular mechanisms of acidic amino acid release from mossy fiber terminals of rat cerebellum. In: "Molecular Mechanisms of Neuronal Responsiveness", Adv. Exp. Med. Biol. 221:237-252.

3. Terrian, D.M., Green, C.L., Dorman, R.V. and Wu, P.H. (1987) Uptake, exchange and release of GABA by cerebellar glomeruli. Neurochem. Res. 12:399-408.

4. Terrian, D.M., Rea, M.A. and Dorman, R.V. (1988) Relationship between prostaglandin synthesis and release of acidic amino acid neurotransmitters. Aviation, Aerospace and Environ. Med. 59:42-49.

5. Dorman, R.V., Schwartz, M.A. and Terrian, D.M. (1988) Depolarization-induced [^{3}H]arachidonic acid accumulation: effects of external Ca^{2+} and phospholipase inhibitors. Brain Res. Bull. 21:445-450.

6. Dorman, R.V. and Terrian, D.M. (1989) The role of arachidonic acid and prostaglandins in the release of neurotransmitters from isolated cerebellar glomeruli. NYAS: Arachidonic Acid Metabolism in the Nervous System (in press).

7. Freeman, E.J., Terrian, D.M. and Dorman, R.V. (1989) Arachidonic acid stimulation of endogenous glutamate release from cerebellar and hippocampal mossy fiber synaptosomes. (in preparation).

8. Damron, D.S. and Dorman, R.V. (1989) Effects of arachidonic acid on calcium mobilization in hippocampal mossy fiber synaptosomes. (in preparation).

9. Dorman, R.V. (1989) Prostaglandin production by cerebellar mossy fiber synaptosomes. (in preparation).

Abstacts:

1. Schwartz, M.A. and Dorman, R.V. (1986) Arachidonic acid metabolism in isolated cerebellar glomeruli. Neurosciences Abstracts 12:825.

2. Terrian, D.M., Rea, M.A. and Dorman, R.V. (1987) Involvement of eicosanoids in the potentiation of D-[^{3}H]aspartate release by phorbol esters. J. Neurochem. (suppl.) 48:S85.

3. Dorman, R.V. (1988) The role of membrane lipids in the evoked release of neurotransmitters from cerebellar glomeruli. AFOSR Review of Basic Research in Neurosciences, p. 23.

4. Dorman, R.V. and Terrian, D.M. (1988) The role of arachidonic acid and prostaglandins in neurotransmitter release from isolated cerebellar

glomeruli. NYAS meeting: Arachidonic Acid in the Nervous System: Physiological and Pathological Significance, p. 7.

5. Dorman, R.V. (1988) Effects of depolarization and Ca^{2+} influx on prostaglandin production in isolated cerebellar glomeruli. Neuroscience Abstracts 14:7.

6. Freeman, E.J., Terrian, D.M. and Dorman, R.V. (1989) Arachidonate evoked release of glutamate from mossy fiber terminals. Trans. Amer. Soc. Neurochem. (in press).

7. Damron, D.S. and Dorman, R.V. (1989) Calcium mobilization in hippocampal mossy fiber terminals. Trans. Amer. Soc. Neurochem. (in press).

PROFESSIONAL PERSONNEL

1. Monica A. Schwartz
   Received M.S. in Biological Sciences, Dec. 19, 1986
   Thesis Title: Arachidonic Acid Metabolism in Isolated Cerebellar Glomeruli

2. Nancy L. Edgehouse
   July 1988 - present; Research Technician

3. Deborah Zetts
   March 1987 - present; Research Technician

4. Ph.D. Students receiving partial support: D. Separovic, E. Freeman, D. Damron and T. Hamm

INTERACTIONS

1. Dorman, R.V., Organizer and Chairman of Symposium/Workshop titled: "Use and Misuse of Nerve Ending Preparations", Amer. Soc. Neurochem., Montreal, Quebec, March 1986.

2. Dorman, R.V. Honors Day Seminar in Dept. PERD, Kent State Univ., Title: "Lipid Metabolism in Cerebellar Glomeruli", April 1986.

3. Presented at AFOSR review, Nov. 29 - Dec. 2, 1987.

4. Dorman, R.V., Biochemistry Seminar: "Prostaglandin involvement in evoked neurotransmitter release, KSU, 11/18/87

5. Dorman, R.V. and Terrian, D.M., presentation at research colloquium, School of Biomedical Sciences, KSU: "Arachidonic acid and neurotransmitter release", 10/20/88

6. Dorman, R.V., seminar at Denison Univ.: "Mechanisms of neurotransmitter release", 11/7/88.

7. Collaborative efforts with DoD laboratory: RVD interacts on a continuing basis with Dr. David M. Terrian, USAF/SAM. This has included frequent visits to SAM by RVD and to Kent State Univ. by DMT. The purpose of these trips was to initiate specific experiments and write manuscripts.